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CLAIMS

(amended; clean version)

1. Method for the microbiological production of α-L-aspartyl-L-phenylalanine (Asp-Phe) from the substrates L-aspartic acid (L-Asp) and L-phenylalanine (L-Phe) characterised in that the substrates are contacted, in the presence of an effective amount of adenosine-triphosphate (ATP), with a non-ribosomal dipeptide synthetase comprising two minimal modules connected by one condensation domain wherein the N-terminal module of these modules is recognising L-aspartic acid and the C-terminal module of these modules is recognising L-phenylalanine and is covalently bound at its N-terminal end to the condensation domain, and wherein each of these minimal modules is composed of an adenylation domain and a 4'-phosphopantetheinyl cofactor containing thiolation domain, and that the α-L-aspartyl-L-phenylalanine (Asp-Phe) formed is recovered.
2. Method for the production of Asp-Phe according to claim 1, characterised in that the condensation domain in the dipeptide synthetase is connected to both minimal modules in such way that it is also covalently bound to the module recognising L-aspartic acid.
3. Method for the production of Asp-Phe according to claim 1 or 2, characterised in that also a thioesterase-like releasing factor is present for the Asp-Phe formed on the dipeptide synthetase.

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4. Method for the production of Asp-Phe according to any of claims 1 to 3, characterised in that the thioesterase-like releasing factor forms an integrated domain of the dipeptide synthetase at the C-terminus thereof.

5. Method for the production of Asp-Phe according to any of claims 1 to 4, characterised in that also a non-integrated protein with thioesterase Type-II-like activity is present together with the dipeptide synthetase.

6. Method for the production of Asp-Phe according to any of claims 1 to 5, characterised in that the dipeptide synthetase is present in living cell-material of a micro-organism, and that glucose, L-Asp and/or L-Phe are being fed to said fermentor, and that the Asp-Phe formed is recovered.

7. Method for the production of Asp-Phe according to claim 6 characterised in that the micro-organism is first grown in a fermentor to reach a predetermined cell density before the expression of the Asp-Phe dipeptide synthetase is switched on and feeding of the glucose, L-Asp and/or L-Phe for the synthesis of the Asp-Phe dipeptide is started.

8. Method for the production of Asp-Phe according to claim 7, characterised in that the micro-organism is an L-phenylalanine producing micro-organism and that only glucose and L-Asp are being fed.

9. Method for the production of Asp-Phe according to claim 8, characterised in that the micro-organism is an Escherichia or Bacillus species.

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10. Method for the production of Asp-Phe according to any of claims 6 to 9, characterised in that the micro-organism used is a strain with reduced protease activity for Asp-Phe or lacking such activity towards Asp-Phe.

11. Method for the production of Asp-Phe according to any of claims 1 to 5, characterised in that the production of Asp-Phe is carried out *in vitro* in an enzyme reactor, while ATP is supplied, and L-Asp and/or L-Phe are being fed, and the Asp-Phe formed is recovered.

12. Method for the production of Asp-Phe according to claim 11, characterised in that the supply of ATP is provided in part by an in situ ATP-regenerating system.

13. Method for the production of Asp-Phe according to claim 12, characterised in that the ATP-regenerating system is present in a permeabilised micro-organism.

14. A DNA fragment or a combination of DNA fragments coding for a non-ribosomal Asp-Phe dipeptide synthetase, which synthetase comprises two minimal modules connected by one condensation domain wherein the N-terminal module of these modules is recognising L-aspartic acid and the C-terminal module of these modules is recognising L-phenylalanine and is covalently bound at its N-terminal end to the condensation domain, and wherein each of these minimal modules is composed of an adenylation domain and a 4'-phosphopantetheinyl cofactor containing thiolation domain.

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15. A DNA fragment coding for an Asp-Phe dipeptide synthetase according to claim 14, characterised in that the condensation domain in the encoded dipeptide synthetase is connected to both minimal modules in such way that it is also covalently bound to the module recognising L-aspartic acid.
16. A DNA fragment according to claim 14 or 15, or a combination of DNA fragments according to claim 14, characterised in that the DNA fragment or combination of DNA fragments encoding the dipeptide synthetase also code for a thioesterase-like releasing factor for the Asp-Phe formed on that dipeptide synthetase.
17. A DNA fragment or a combination of DNA fragments according to claim 16, characterised in that the thioesterase-like releasing factor forms an integrated domain of the dipeptide synthetase at the C-terminus thereof.
18. A DNA fragment or a combination of DNA fragments according to any of claims 14 to 17, characterised in that it/they also code for a non-integrated protein with thioesterase Type-II-like activity.
19. A recombinant micro-organism containing a DNA fragment or a combination of DNA fragments according to any of claims 14-18.
20. A micro-organism according to claim 19 wherein the micro-organism is capable of producing L-Asp and/or L-Phe.
21. A micro-organism according to claim 20 wherein the micro-organism is an *Escherichia coli* or *Bacillus* species.

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22. Asp-Phe dipeptide synthetase characterised in that it comprises two minimal modules connected by one condensation domain wherein the N-terminal module of these modules is recognising L-aspartic acid and the C-terminal module of these modules is recognising L-phenylalanine and is covalently bound at its N-terminal end to the condensation domain, and wherein each of these minimal modules is composed of an adenylation domain and a 4'-phosphopantetheinyl cofactor containing thiolation domain.

23. Asp-Phe dipeptide synthetase according to claim 22 characterised in that the condensation domain in the dipeptide synthetase is connected to both minimal modules in such way that it is also covalently bound to the module recognising L-aspartic acid.

24. Asp-Phe dipeptide synthetase according to claim 22 or 23, characterised in that the dipeptide synthetase also comprises a thioesterase-like releasing factor for the Asp-Phe formed on that dipeptide synthetase.

25. Asp-Phe dipeptide synthetase according to claim 24, characterised in that the thioesterase-like releasing factor forms an integrated domain of the dipeptide synthetase at its C-terminus.

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